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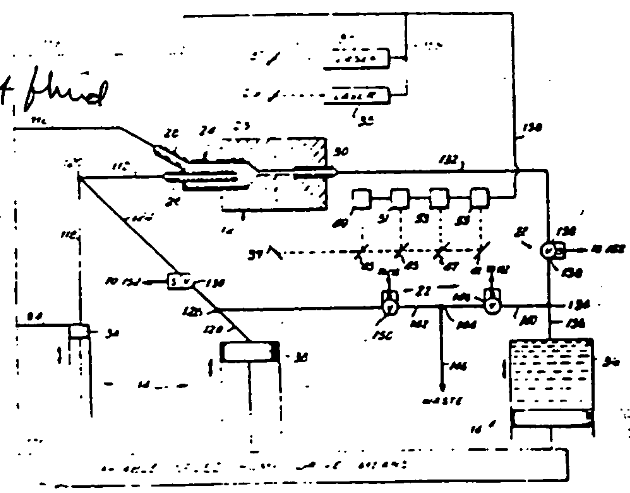
(54) Apparatus and method for supply of sample and sheath liquids to analytical flow cell.

(57) An apparatus and method, especially useful in automated analytical systems, for concomitant pumping, to a sheath stream flow cell (12), a first fluid (e.g. sample) from a selected one of a plurality of different sources (40, 42, 44) and a second fluid (e.g. sheath) from a selected one of a plurality of different sources (96, 98, 100), including control means (22, 152) to select appropriate first and second fluids from said different sources. Differential pumping is preferably employed.

1st fluid



EP 0 107 333 A2

APPARATUS AND METHOD FOR SUPPLY OF SAMPLE AND SHEATH LIQUIDS TO ANALYTICAL FLOW CELL

This invention relates to an apparatus and method for the precisely controlled and coordinated supply of sheath stream and sample fluids to sheath stream flow cell systems, such as high-speed automated biomedical analytical systems.

Although apparatus and methods are known for the supply of sheath stream and sample fluids to sheath stream flow cells, none accomplish these functions in a precisely controlled and coordinated manner using the same sheath stream flow cell, and associated detecting and pumping means, for successive different types of sample analyses.

In many instances in the prior art, peristaltic pumping is used to supply the sample fluid stream to the flow cell. This is disclosed, for example, in United States Patent 3,740,143, wherein multi-channel peristaltic pumping is used to supply respective series of diluted blood samples to a plurality of sheath stream flow cells for a different type of sample analysis with respect to each. This leads to less than optimal accuracy in cell analyses due to marginal variations in peristaltic pump roller and pump tube dimensions, which cause variations in the diameter, velocity and/or volume of the sample fluid stream through the flow cell. Since separate pumping systems are used, with the sheath stream fluid being pressure pumped from a constantly pressurized source, variations in the essential sheath-

vacuum pumping, requiring liquid trap, pressure regulation, pressure gauge, and needle valve or other flow restrictor means, to those ends. These apparatus and methods can be difficult to calibrate and tend not to remain calibrated, and thus have also not proven fully satisfactory, especially in increasingly sophisticated automated biomedical analytical systems. Again, a separate sheath stream flow cell, and associated detecting and pumping means, are required for each type of sample analysis to be performed.

An apparatus and method for the precisely controlled and coordinated supply of sample and sheath stream fluids under optimal conditions to maximize the accuracy and reproducibility of successive sample analyses are disclosed in our copending European patent application no. 83303532.2 Here too, however, a separate sheath stream flow cell and associated detecting and pumping means are required for each different type of sample analysis to be performed.

Sheath stream flow cell analysis apparatus utilizing a selector valve to permit somewhat different types of sample analyses by the same sheath stream flow cell is known in the form of the hematology instrument "Ortho ELT8" as manufactured by Ortho Diagnostic Systems, Inc. of Westwood, Massachusetts. However, this instrument utilizes independent and complex sample fluid supply channels, rather than separate sample fluid sources, for different sample analyses, thereby requiring at least one separate sample fluid pump for each sample fluid analysis of interest, with resultant increase in the overall complexity, cost and maintenance requirements of the apparatus. This also increases sample carryover. Also, no provision is made in this apparatus for the selection and supply of different sheath stream fluids to the flow cell whereby successive markedly different types of sample analyses, requiring in turn markedly different types of respectively optically compatible sheath stream

fluids, cannot be performed thereon. Also, no provision is made in this apparatus for the precisely coordinated and controlled differential pumping of the sample and sheath fluids to the flow cell, thus rendering extremely difficult, if not impossible, the true optimization of the accuracy and reproducibility of the sample analysis results.

We have now devised an apparatus and method whereby a sample, from one of a number of different sample sources, is selected and pumped to a sheath stream flow cell concomitantly with an appropriate sheath stream from one of a number of different sources thereof. Thus, the invention provides a random access single channel sheath stream apparatus and method for the precisely controlled and coordinated supply of sheath stream and sample fluids from respective pluralities of different sources thereof to a common sheath stream flow cell for sample fluid analyses, thereby optimizing the accuracy and reproducibility of those analyses results.

According to the invention, there is provided apparatus for the concomitant supply of a first fluid, from a selected one of a plurality of separate sources of different first fluids, and a second fluid, from a selected one of a plurality of separate sources of different second fluids, respectively, to a sheath stream flow cell analysis means which includes a sheath stream flow cell, which apparatus comprises flow directing and control means operatively associated with each of said separate first and second fluid sources, and said sheath stream flow cell, respectively, said flow directing and control means being operable to connect the selected ones, only, of said separate first and second fluid sources, and said sheath stream flow cell, for concomitant flow of said selected first and second fluids through said sheath stream flow cell.

The invention also includes a method for the concomitant supply of a first fluid from a selected one of a

plurality of separate sources of different first fluids, and a second fluid from a selected one of a plurality of separate sources of different second fluids, respectively, to sheath stream flow cell analysis means which includes a sheath stream flow cell, which method comprises connecting the selected one, only, of said plurality of first fluid sources to said sheath stream flow cell, and concomitantly connecting the selected one, only, of said plurality of second fluid sources to said sheath stream flow cell, thereby enabling concomitant flow of said selected first and second fluids through said sheath stream flow cell.

The apparatus and method of the invention are particularly adapted for different types of differentiation and counting of sample particles, through use of the same sheath stream flow cell, and associated detecting and pumping means, thereby materially reducing apparatus cost and complexity over prior art proposals. In addition, the apparatus and method are operable with minimal, if any, damage to the sample particles, and with greatly reduced sample carryover when successive samples are supplied, in turn, to the sheath stream flow cell for sequential sample analyses. The apparatus and method are operable at extremely high sample analyses rates, and are particularly versatile in operation, being very readily adapted for use with wide ranges of different sample and sheath stream liquids attendant the performance of a wide variety of different analyses on the sample liquids.

The random access single channel sheath stream apparatus and method of the invention enable the supply of sample and sheath liquids to a sheath stream flow cell for successive, different types of sample analyses, and comprise a plurality of separate sources for differently reacted (for example) sample liquid portions, and a plurality of separate sources of different, respectively optically compatible sheath liquids. Preferably, variable speed sample

and sheath liquid pumps are connectable to selected ones of said sample and sheath liquid sources by flow directing and control means, and are operable preferably to differentially pump, by concomitantly pumping and aspirating, the selected sample and sheath liquids through a single channel, e.g. common flow cell, at optimal, precisely coordinated flow rates to maximize the accuracy and reproducibility of the sample analyses results in each instance. A shuttle pump is preferably provided to rapidly prime the sample liquid pump and thereby increase the operational speed of the apparatus. The use of the same sheath stream flow cell for different types of sample analyses significantly reduces the complexity and cost of the apparatus. The apparatus find particularly useful application in high-speed, automated biomedical analytical systems.

In order that the invention may be more fully understood, an embodiment thereof will now be described with reference to the accompanying drawing wherein FIGURES 1A and 1B together show a generally schematic diagram of one form of apparatus of the invention, depicted with a sheath stream flow cell.

Referring now to FIGURES 1A and 1B, the random access single channel sheath stream apparatus 10 of the invention is shown with a sheath stream flow cell 12. Apparatus 10 comprises pump means 14 which supply selected sheath and sample fluids for concomitant flow through flow cell 12 for sample fluid analysis. Variable speed pump drive means are indicated schematically at 16. Plural sample and sheath fluid sources are indicated generally at 18 and 20, respectively. Flow directing and control means 22 comprise a plurality of solenoid or other valve-controlled conduits which operate to select sample and optically com-atible sheath fluids from the respective plural sources thereof for concomitant flow through flow cell 12 as directed by an apparatus controller 152.

Sheath stream flow cell 12 may, for example, generally take the form of that disclosed in United States Patent 3,661,460, to which reference should be made for further details. Flow cell 12 comprises a body 23 and concentric flow module 24 with the latter including a sample stream inlet 26, sheath stream inlet 28, and a mixed stream outlet 30. Although not, _per se_, forming part of this invention, it may be understood that the sheath stream flow cell 12 brings the sample and sheath streams introduced at inlets 26 and 28, respectively, together to form a pair of concentric, substantially unmixed streams, with the sample stream at the centre. Detecting and counting means 32 comprises lasers 33 and 35 of different wavelengths, mirrors 37, 39 and 41, dichroic mirrors 29, 43, 45 and 47, and detectors 49, 51, 53 and 55, operatively arranged relative to flow cell 12. The detecting and counting means 32 is operative to count and size particles per unit

volume of the sample stream as the ensheathed stream flows through the flow cell body 23. Precise control of the velocity, stability and diameter of the sample stream provide for a precise counting and sizing process.

Pump means 14 comprises sample fluid pump 34, sheath fluid pump 36, and shuttle pump 38; each of which is preferably a syringe pump of the type manufactured by the Hamilton Company of Reno, Nevada. Such pumps are calibratable and traceable to the particularly demanding standards of the National Bureau of Standards and, once satisfactorily calibrated, tend to remain so for long periods of operational time. Pump drive means 16 preferably take the form of precisely controlled stepping motors which may be microprocessor controlled in accordance with an oscillator-generated time base. Such components are manufactured by the Superior Electric Company of Bristol, Conn. This pump and pump drive means combination provides for precise control of the respective pump flow rates and flow volumes. Preferably, shuttle pump 38, sheath fluid pump 36 and pump drive means 16 are arranged so that pumps 38 and 36 are 180° out of phase. Thus, for example, with pump 38 at top dead center, pump 36 will be at bottom dead center and vice versa.

Sample fluid sources 18 comprise reaction vessels 40, 42 and 44, respectively. Samples and reagents supplier 46, which may take any appropriate known form supplies sample and reagent fluids to reaction vessels 40, 42 and 44 through conduits 48, 50 and 52, respectively.

Constantly pressurized wash liquid reservoir 54 supplies a wash liquid to reaction vessels 40, 42 and 44 through conduits 56, 58 and 60, respectively. Solenoid operated valves 62, 64 and 66 are respectively disposed along conduits 56, 58 and 60 and are operable to permit or prevent wash liquid flow therethrough.

Reaction vessels 40, 42 and 44 respectively include drain conduits 68, 70 and 72 extending downwardly from the vessel bottoms to vacuum; and solenoid operated valves 74, 76 and 78 are respectively disposed along drain conduits 68, 70 and 72 to permit or prevent flow therethrough. Conduits 80, 82 and 84 respectively connect drain conduits 68, 70 and 72 as shown, above the drain conduit valve in each instance, to four way junction 86; and solenoid operated valves 88, 90 and 92 are respectively disposed along conduits 80, 82 and 84 to permit or prevent flow therethrough. Conduit 94 connects junction 86 to sample pump 34 and supplies reacted sample fluids thereto.

Sheath fluid sources 20 comprise constantly pressurized sheath fluid reservoirs 96, 98 and 100, respectively. Conduits 102, 104 and 106 respectively connect the sheath fluid reservoirs 96, 98 and 100 to four way junction 108; and solenoid operated valves 110, 112 and 114 are respectively disposed along conduits 102, 104 and 106 to permit or prevent flow therethrough. A sheath fluid supply conduit 116 connects junction 108 to sheath stream inlet 28 of the sheath stream flow cell 12.

Conduit 118 connects sample pump 34 to three way

junction 120, and reacted sample supply conduit 122 connects junction 120 to sample stream inlet 26 of the sheath stream flow cell 12. Conduit 124 connects junction 120 to three way junction 126, and conduit 128 connects the latter to shuttle pump 38. Solenoid operated valve 130 is disposed along conduit 124 to permit or prevent flow therethrough.

Conduit 132 connects mixed stream outlet 30 of the flow cell 12 to three way junction 134, and conduit 136 connects the latter to sheath fluid pump 36. A solenoid operated valve 138 is disposed along conduit 132 to permit or prevent flow therethrough. Conduits 140 and 142 respectively connect junctions 134 and 126 to three way junction 144, and conduit 146 connects junction 144 to waste. Solenoid operated valves 148 and 150 are respectively disposed along conduits 140 and 142 to permit or prevent flow therethrough

Controller 152 is operable through connectors 154, 156 158 and 159 to control and coordinate the respective operations of samples and reagents supplier 46, pump drive means 16, and detecting and counting means 32. In addition, controller 152 is operable as indicated to control and coordinate the respective operations of the solenoid operated valves 62, 64, 66, 74, 76, 78, 88, 90, 92, 110, 112, 114, 130, 138, 148 and 150 of the flow directing and control means 22. Where the apparatus conduits are resilient tubing, the respective solenoid operated valves may be pinch valves.

A representative application of the apparatus and method of this invention is as a hematology instrument for the automated counting and sizing of the red blood cells and platelets, the basophils, and all of the other white blood

cells except basophils, as are respectively contained in a series of diluted blood sample portions which are supplied in turn, each with an appropriate reagent quantity, to the reaction vessels 40, 42 and 44 by samples and reagents supplier 46 in timed sequence with overall apparatus operation as determined by controller 152. In such instance, sheath fluid reservoirs 96, 98 and 100 would respectively contain a sheath liquid which is optically compatible with red blood cell and platelet counting and sizing, a sheath liquid which is optically compatible with basophil counting and sizing, and a sheath liquid which is optically compatible with the counting and sizing of all other white blood cells except basophils.

For such application, appropriate quantities of the reacted sample portions of the same blood sample from reaction vessels 40, 42, and 44 with, in each instance, an appropriate quantity of the selected, refractive index-compatible sheath liquid from one of the reservoirs 96, 98 and 100, are concomitantly pumped in turn, as directed by flow control means 22, to and through the same sheath stream flow cell 12 by pump means 14 for respective red blood cell and platelet, basophil, and remaining white blood cell counting and sizing by detecting and counting means 32, all in timed sequence as determined by controller 152. The remaining, reacted blood sample portions are then emptied from the reaction vessels 40, 42 and 44; and the reaction vessels are then washed by wash liquid from reservoir 54 to minimize sample carryover (the contamination of a succeeding sample by the residue of a preceding sample), again in timed sequence as determined by controller 152. Thereafter, portions of the next

succeeding blood sample, with appropriate reagent quantities in each instance, are supplied to reaction vessels 40, 42 and 44 by samples and reagents supplier 46 for repetition of the cell counting and sizing process.

More specifically, at the beginning of an operating sequence, each of reaction vessels 40, 42 and 44 contain appropriately reacted portions of the same blood sample, pressurized sheath reservoirs 96, 98 and 100 contain appropriate quantities of refractive index-compatible sheath liquids, shuttle pump 38 and sample pump 34 are at top dead center (as shown), and sheath pump 36 is at bottom dead center and filled with preceding sample and sheath liquids (as shown). All solenoid operated valves are closed, and all conduits are liquid filled except for drain conduits 68, 70 and 72. Opening of valves 110, 150 and 138 by controller 152 will flow pressurized sheath liquid from reservoir 96 through conduit 102, junction 108, conduit 116, flow cell inlet 28, flow cell 12, flow cell outlet 30, conduit 132, junction 134, and conduits 140 and 146 to waste. This purges the flow cell of the residue of the preceding sample and sheath liquids to minimize sample and sheath liquid carryover and maximize analysis accuracy; it being well understood by those skilled in this art that sample carryover in the interior optical window of flow cell flow chamber 24 can be particularly detrimental to optical counting of low signal level sample liquid particles. Controller 152 then closes valves 110, 150 and 138.

Controller 152 then opens valves 88, 130 and 150, and the piston of shuttle pump 38 is driven down by pump drive means 16 to rapidly aspirate the segment of air which is trapped by surface tension in drain conduit 68 above valve 74, followed

by the reacted sample from reaction vessel 40, through conduit 80, junction 86, conduit 94, sample pump 34, conduit 118, junction 120, conduit 124, junction 126 and conduit 128. This purges the preceding sample from the sample pump 34 and conduits 80, 94 and 118 to further minimize sample carryover, and washes in the reacted sample from vessel 40 substantially to the inlet of flow cell 12. Concomitantly, the piston of sheath fluid pump 36 is driven up by pump drive means 16 to rapidly discharge its contents of sample and sheath liquids from the preceding analyses to waste through conduit 136, junction 134, conduit 140, junction 144 and conduit 146. The piston of sample fluid pump 34 is then driven down by pump drive means 16 to aspirate reacted sample liquid from reaction vessel 40 through drain conduit 68, conduit 80, junction 86 and conduit 94. Controller 152 then closes valves 88, 130 and 150.

With the desired sample so-positioned, controller 152 opens valves 110, 148 and 138. The piston of sample fluid pump 34 is driven up by pump drive means 16 to positively pump, at a precisely controlled optimal flow rate, the pump-contained reacted sample liquid from reaction vessel 40 through the flow cell 12 through conduit 118, junction 120, conduit 122 and flow cell inlet 26. Concomitantly, the piston of sheath fluid pump 36 is driven down by pump drive means 16 to aspirate, also at precisely controlled optimal flow rate, the optically compatible sheath liquid from reservoir 96 through the flow cell 12 through conduit 102, junction 108, conduit 116, flow cell sheath inlet 28, flow cell 12, flow cell outlet 30, conduit 132, junction 134 and conduit 136.

In addition, the reacted sample liquid from vessel 40, as pumped through flow cell 12 by sample fluid pump 34, is also aspirated into sheath fluid pump 36 from the flow cell outlet 30. This forms the concentric sample-sheath liquid streams through the flow cell under precisely controlled and coordinated, readily reproducible conditions of constant, and optimal, sample and sheath liquid stream diameters, velocity and flow volumes in respect to the sample liquid from reaction vessel 40. Once steady state sheath stream flow conditions through flow cell 12 are reached, controller 152 activates the appropriate one of lasers 33 and 35, and the appropriate one of detectors 49, 51, 53 and 55 (both in accordance with the particular characteristics of the sample analysis of interest) for a precisely predetermined time period of cell counting and sizing operation; which time period is the same for all sample liquids from reaction vessel 40.

With sheath fluid pump 36, sample fluid pump 34, and the respective sheath liquid reservoirs 96, 98 and 100 configured and operatively connected as described through the sheath stream flow cell 12, it will be clear that a precisely operable and coordinated differential pumping arrangement is provided. More specifically, with incompressible fluids, the flow rate at which the sheath liquid is aspirated as described by sheath fluid pump 36 will be precisely equal to the difference between the total flow rate into sheath fluid pump 36 and the flow rate at which the sample liquid is pumped as described from sample fluid pump 34. This ensures that the flow rate of the sheath liquid through the sheath stream flow cell 12 will be precisely equal to the difference between the total flow rate through the flow cell and the flow rate

of the sample liquid therethrough. Thus, with a constant and precisely determinable total flow rate into sheath fluid pump 36, it will be clear that precise determination and control of the sample liquid flow rate out of sample fluid pump 34 to and through the sheath stream flow cell 12 will operate in turn to precisely determine and control the sheath liquid flow rate through the flow cell 12; whereby the essential sheath and sample liquid stream diameters, flow rates and flow velocities through the flow cell 12, and the respective ratios therebetween, may be optimized and maintained consistent for each particular type of sample analysis of interest. This maximizes the accuracy and reproducibility of the sample analysis results.

Simultaneously with cell counting and sizing, the piston of shuttle pump 38 is driven up by drive means 16 to discharge its contents of the reacted sample liquid from reaction vessel 40 through conduit 128, junction 126, conduit 142, junction 144 and conduit 146 to waste. Controller 152 then closes valves 110, 148 and 138.

To begin analysis of the reacted sample portion from reaction vessel 42, controller 152 opens valves 112, 150 and 138 allowing sheath liquid from reservoir 98 to purge flow cell 12 by flowing through conduit 104, junction 108, conduit 116, flow cell inlet 28, flow cell 12, flow cell outlet 30, conduit 132, junction 134, conduit 140, junction 144 and conduit 146 to waste. Controller 152 then closes valves 112, 150 and 138.

Valves 90, 130 and 150 are then opened by controller 152, and the piston of shuttle pump 38 driven down by drive

means 16 to rapidly aspirate the segment of air drain conduit 70 followed by the reacted sample liquid from reaction vessel 42 through conduit 82, junction 86, conduit 94, sample fluid pump 34, conduit 118, junction 120, conduit 124, junction 126 and conduit 128. This purges the residue of the preceding sample from reaction vessel 42 from conduit 82, and purges the residue of the preceding sample from reaction vessel 40 from junction 86, conduit 94, pump 34, conduit 118, junction 120 and conduit 124, all to further minimize sample carryover. This also washes in the reacted sample liquid from reaction vessel 42 substantially to flow cell sample inlet 26. Concomitantly, the piston of sheath fluid pump 36 is driven up by pump drive means 16 to rapidly discharge its contents of sample and sheath liquids from the preceding analysis of the reacted sample liquid from reaction vessel 40 through conduit 136, junction 134, conduit 140, junction 144 and conduit 146 to waste.

The piston of sample pump 34 is then driven down by pump drive means 16 to aspirate reacted sample liquid from reaction vessel 42 through drain conduit 70, conduit 82, junction 86 and conduit 94. Controller 152 then closes valves 90, 130 and 150. With the desired sample so positioned, controller 152 opens valves 112, 148 and 138. Sample pump 34 is then driven up by pump drive means 16 to positively pump, at a precisely controlled optimal flow rate, the pump-contained reacted sample liquid from reaction vessel 42 through flow cell 12 through conduit 118, junction 120, conduit 122 and the flow cell inlet 12. Concomitantly, the piston of sheath fluid pump 36 is driven down by pump drive means 16 to aspirate, also at a precisely controlled optimal flow rate, the optically

compatible sheath liquid from reservoir 98 through conduit 104, junction 108, conduit 116, flow cell sheath inlet 28, flow cell 12, flow cell outlet 30, conduit 132, junction 134 and conduit 136. In addition, the reacted sample liquid from vessel 42 as pumped through the flow cell 12 by sample fluid pump 34 is also aspirated into sheath fluid pump 36 from flow cell outlet 30. This forms the concentric, sample-sheath liquid streams through flow cell 12 under precisely controlled and coordinated, readily reproducible conditions of constant, and optimal, sample and sheath liquid stream diameters, velocity and flow volumes in respect to the reacted sample liquid from reaction vessel 42. As with the previous sample, once steady state sheath stream flow conditions through flow cell 12 are reached, controller 152 activates the appropriate ones of the lasers 33 and 35, and the detectors 49, 51, 53 and 55, respectively, for a precisely predetermined timed period of cell counting and sizing operation which is the same for all reacted sample liquids from reaction vessel 42.

Simultaneously with this cell counting and sizing, the piston of shuttle pump 38 is again driven by drive means 16 to discharge its contents of the reacted sample liquid from reaction vessel 42 through conduit 128, junction 126, conduit 142, junction 144 and conduit 146 to waste. Controller 152 then closes valves 112, 148 and 138.

To begin analysis of the reacted sample liquid portion from reaction vessel 44, controller 152 opens valves 114, 150 and 138 allowing sheath liquid from reservoir 100 to purge flow cell 12 as previously described, whereupon controller 152 closes those valves. Next, controller 152 opens valves

92, 130 and 150, and the piston of shuttle pump 38 is driven down by pump drive means 16 to rapidly aspirate the segment of air from drain conduit 72 followed, by reacted sample liquid from reaction vessel 44, through drain conduit 72, conduit 84, junction 86, conduit 94, sample fluid pump 34, conduit 118, junction 120, conduit 124, junction 126 and conduit 128. This purges the preceding sample residue from sample pump 34 and the relevant conduits and junctions, and washes in the reacted sample liquid from reaction vessel 44 substantially to the flow cell inlet 26. Concomitantly, the piston of sheath fluid pump 36 is driven up by pump drive means 16 to rapidly discharge its contents of sample and sheath liquids from the preceding analysis to waste as described above.

The piston of sample pump 34 is then driven down by pump drive means 16 to aspirate reacted sample liquid from reaction vessel 44 through drain conduit 72, conduit 84, junction 86 and conduit 94. Controller 152 then closes valves 92, 130 and 150. With the desired sample so-positioned, controller 152 opens valves 114, 148 and 138. The piston of sample fluid pump 34 is then driven up by pump drive means 16 to positively pump, at a precisely controlled optimal flow rate, the pump-contained reacted sample liquid from reaction vessel 44 to and through the flow cell inlet 26. Concomitantly, the piston of sheath fluid pump 36 is driven down by pump drive means 16 to aspirate, also at precisely controlled optimal flow rate, the optically compatible sheath liquid from reservoir 100 to and through the flow cell 12 through conduit 106, junction 108, conduit 116, flow cell sheath inlet 28, flow cell 12, flow cell outlet 30, conduit 132, junction 134 and conduit 136. In addition, the reacted sample liquid from

vessel 44, as pumped through flow cell 12 by sample fluid pump 34, is also aspirated into sheath fluid pump 36 from flow cell outlet 30. This forms the concentric sample-sheath liquid streams through flow cell 12 under precisely controlled and coordinated, readily reproducible conditions of constant, and optimal, sample and sheath liquid stream diameters, velocity and flow volumes in respect to the reacted sample liquid from reaction vessel 44. Again, once steady state sheath stream flow conditions are reached in sheath stream flow cell 12, controller 152 activates detecting and counting means 32 for a precisely predetermined time period of cell counting and sizing operation. This time period is the same for all reacted sample liquids from reaction vessel 44.

Simultaneously with this cell counting and sizing, the piston of shuttle pump 38 is again driven up by pump drive means 16 to discharge its contents of the reacted sample liquid from reaction vessel 44 to waste as previously described. Controller 152 then closes valves 112, 148 and 138 to prepare the apparatus 10 for the next cycle of operation as described.

The time sequence of the supply of the samples and reagents to the respective reaction vessels 40, 42 and 44 by supplier 46 is selected to avoid loss in operational time and thus maximize the sample analysis rate of apparatus 10. As soon as valve 88 is closed by controller 152 following aspiration of reacted sample liquid from reaction vessel 40, the controller opens valve 74 to drain the remainder of the reacted sample liquid in reaction vessel 40 under vacuum through drain conduit 68 to waste. Once this is completed, controller 152 opens valve 62 to supply wash liquid from wash liquid reservoir 54 to reaction vessel 40 to rapidly wash the

residue of the reacted sample liquid therefrom for drain, under vacuum through drain conduit 68 to waste. Valve 62 is then closed by controller 152 whereby, with valve 74 remaining open, ambient air will be drawn to vacuum through the reaction vessel 40 and drain conduit 68 to further, and rapidly, remove reacted sample and wash liquid residues therefrom and dry the vessel. Valve 74 is then closed by controller 152, and samples and reagents supplier 46 activated by the latter to supply an appropriate portion of the succeeding sample, along with an appropriate reagent quantity, to reaction vessel 40 through conduit 48 for commencement of the desired reaction. Thus, no operational time of the apparatus 10 is lost in waiting for this reaction which may proceed to completion during the analysis as described of the preceding reacted sample liquid from reaction vessel 40 or, if more time consuming, certainly during the analyses of the reacted sample liquids from reaction vessels 42 and 44. This overlapping of the sample liquid-reagent reaction and sample liquid analyses times operates to provide a higher operational rate for the apparatus 10 than would otherwise be possible in that an appropriately reacted sample liquid is always immediately available for analysis.

Operation of pressurized wash liquid reservoir 54 and samples and reagents supplier 46 vis-a-vis reaction vessels 42 and 44 through conduits 58, 60, 50 and 52, valves 76 and 78, and drain conduits 70 and 72 is the same as described for reaction vessel 40.

Additional increase in the overall operational rate of apparatus 10 is provided by variable speed pump drive means 16 which enable the rapid washing in or priming of the apparatus, and the rapid establishment of a steady state

sample stream by the shuttle pump 38, and the rapid initial achievement of steady state sample and sheath liquid streams through sheath stream flow cell 12 by sample and sheath fluid pumps 34 and 38; all followed as required by coordinated reduction in sample and sheath fluid pump drive rates to assure optimal stream flow rates through flow cell 12 for respective cell counting and sizing of reacted sample liquids by detecting and counting means 32 from each of reaction vessels 40, 42 and 44. Too, shuttle pump 38 enables the rapid washing in of samples from relatively remote sample locations without sample carryover of significance thus, for example, enabling wide spacing, if desired, between the reaction vessels and the detecting and counting means.

Operation of the apparatus 10 is continuous until all of the samples in the sample series of interest have been analyzed.

Although described as comprising three reaction vessels and three sheath liquid reservoirs, the apparatus 10 is by no means limited to that number.

CLAIMS:

1. Apparatus for the concomitant supply of a first fluid, from selected one of a plurality of separate sources (40,42,44) of different first fluids, and a second fluid, from a selected one of a plurality of separate sources (96,98,100) of different second fluids, respectively, to a sheath stream flow cell analysis means which include a sheath stream flow cell (12), which apparatus comprises flow directing and control means (22) operatively associated with each of said separate first and second fluid sources, and said sheath stream flow cell, respectively, said flow directing and control means being operable to connect the selected ones, only, of said separate first and second fluid sources, and said sheath stream flow cell, for concomitant flow of said selected first and second fluids through said sheath stream flow cell.

2. Apparatus according to claim 1 wherein, said flow directing and control means comprise conduit means (68,72, 80 to 86, 94,118,120,112) connecting said pluralities of said first and second fluid sources and said sheath stream flow cell, and valve means (88,90,92,110,112,114,138,148,150) operatively associated with said conduit means and operable to control fluid flow therethrough for selection of said first and second fluids; and wherein said sheath stream flow cell analysis means comprises detecting means (49,51, 53,55) operatively associated with said sheath stream flow cell, said detecting means being operable to detect respectively different characteristics of said first fluids.

3. Apparatus according to claim 1 or 2, further comprising, pump means (34,36,38) operatively connected to said flow directing and control means and to said sheath stream flow cell and operable to concomitantly pump the

selected ones of said first and second fluids from the respective fluid sources to said sheath stream flow cell for concomitant fluid flow therethrough.

4. Apparatus according to claim 3 which comprises variable speed pump drive means (16) operatively associated with said pump means and operable to drive the latter at selected pumping rates in accordance with first and second fluid selection whereby, the selected ones of said first and second fluids may be concomitantly pumped through said sheath stream flow cell at respectively optimal flow rates.

5. Apparatus according to claim 3 or 4, wherein said pump means (34,36,38) comprises differential pump means operatively connected to the inlet means (26,28) and outlet means (30), respectively, of said sheath stream flow cell, said differential pump means being operable to pump the selected one of the first of said fluids to said sheath stream flow cell inlet means at a first flow rate, and to concomitantly aspirate the selected ones of the first and second of said fluids through said sheath stream flow cell outlet means at a second, and greater flow rate whereby, the flow rate of the second of said fluids through said sheath stream flow cell will be equal to the difference between said second and first flow rates, respectively.

6. Apparatus according to claim 5, which is arranged so that, in use, said first fluids comprise a plurality of sample liquids, and said second fluids comprise a plurality of respectively optically compatible sheath liquids, and wherein said differential pump means comprise a sample liquid pump (34) which is connectable by said flow directing and control means to the selected one of the plurality of sample liquid sources and to the sheath stream flow cell inlet means to pump the selected sample liquid from said

sample liquid source to the sheath stream flow cell inlet means, and a sheath liquid pump (36) which is connectable by said flow directing and control means through the sheath stream flow cell outlet and inlet means, respectively, to the selected one of the plurality of sheath liquid sources to concomitantly aspirate the selected sheath liquid, and the selected sample liquid as pumped by the sample liquid pump, through said sheath stream flow cell.

7. Apparatus according to claim 6, wherein means are provided to pressurise the respective sources of said sheath liquids.

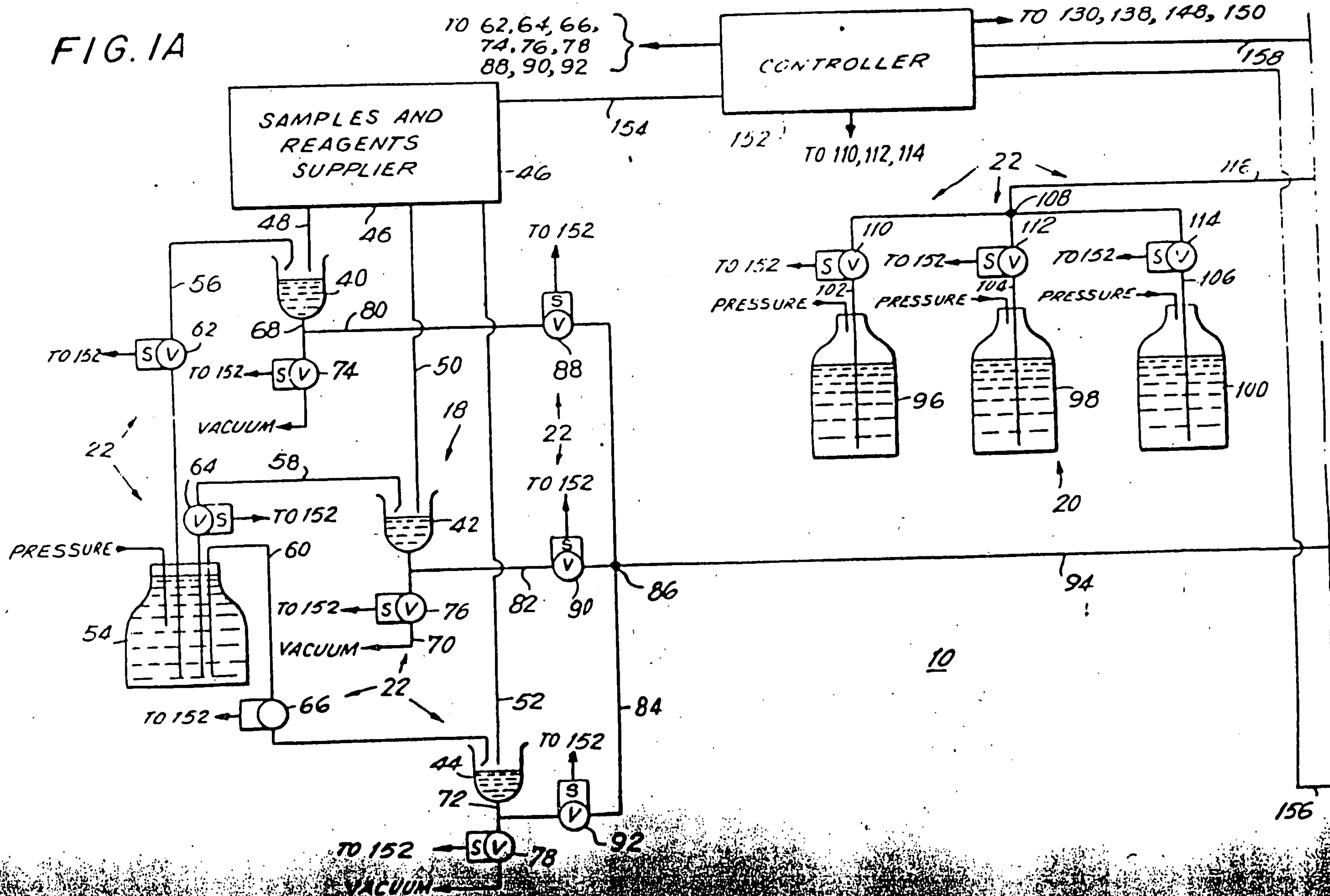
8. A method for the concomitant supply of a first fluid from a selected one of a plurality of separate sources (40,42,44) of different first fluids, and a second fluid from a selected one of a plurality of separate sources (96, 98,100) of different second fluids, respectively, to sheath stream flow cell analysis means which includes a sheath stream flow cell (12), which method comprises connecting the selected one, only, of said plurality of first fluid sources to said sheath stream flow cell, and concomitantly connecting the selected one, only, of said plurality of second fluid sources to said sheath stream flow cell, thereby enabling concomitant flow of said selected first and second fluids through said sheath stream flow cell.

9. A method according to claim 8, further comprising varying the rates at which the selected ones of said first and second fluids are pumped for flow through said sheath stream flow cell in accordance with the selections of said fluids whereby, the selected ones of said first and second fluids may be concomitantly flowed through said sheath stream flow cell at respectively optimal flow rates, and wherein a characteristic of the selected one of

said first fluids is detected, as the same is flowed through said sheath stream flow cell, in accordance with the selection of said fluid.

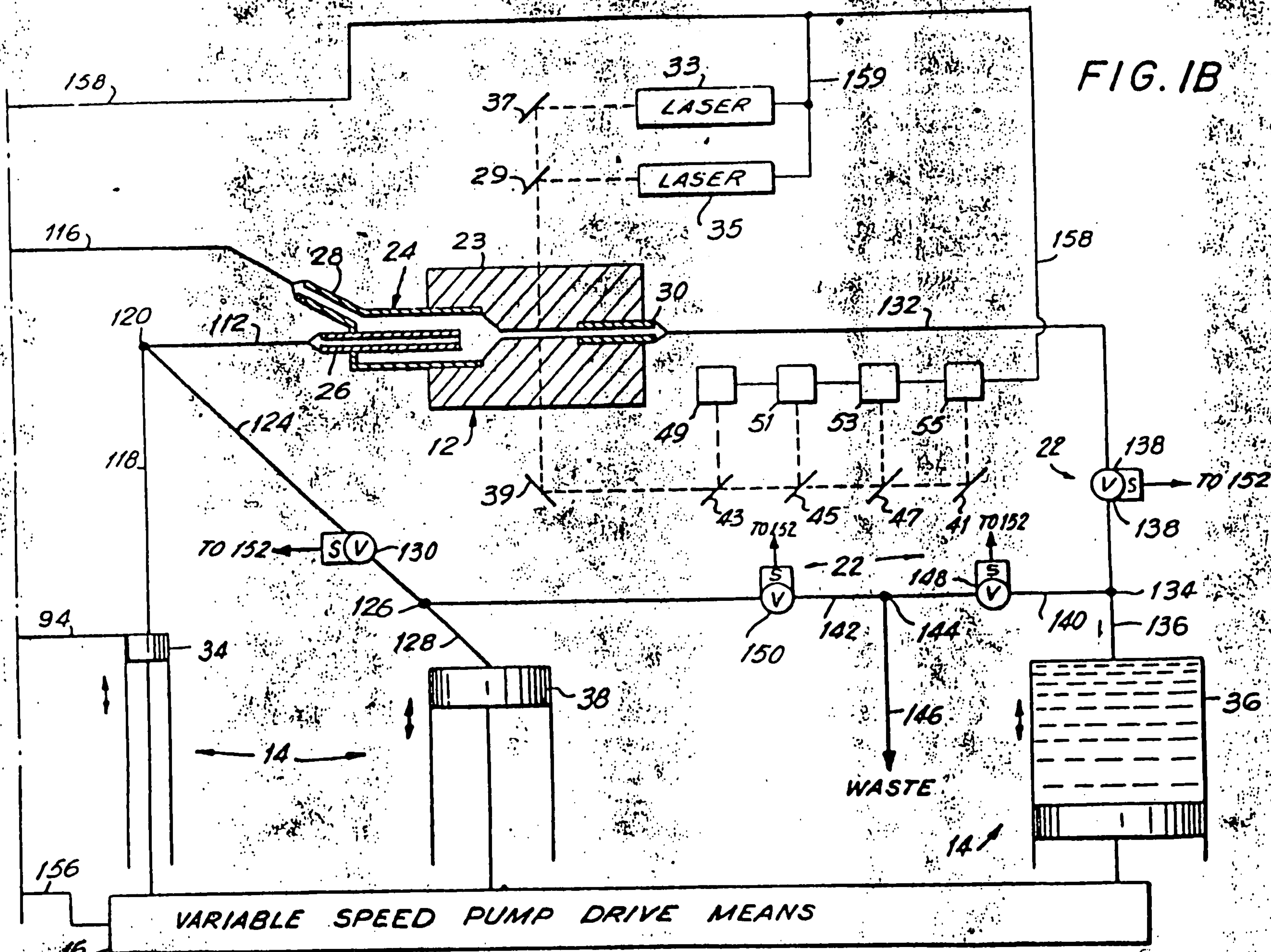
10. A method according to claim 9, wherein the selected one of the first of said fluids is pumped to said sheath stream flow cell at a first flow rate, and the selected ones of the first and second of said fluids are concomitantly aspirated through said sheath stream flow cell at a second, and greater flow rate whereby, the flow rate of the second of said fluids through said sheath stream flow cell is equal to the difference between said second and first flow rates, respectively.

FIG. 1A



CONTROLLER
SAMPLES AND REAGENTS SUPPLIER
TO 62, 64, 66, 74, 76, 78 88, 90, 92
TO 130, 138, 148, 150
TO 110, 112, 114
TO 152
PRESSURE
VACUUM

FIG. 1B


LASER
LASER
TO 152
TO 152
TO 152
TO 152
WASTE
VARIABLE SPEED PUMP DRIVE MEANS